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A non-enzymatic hydrogen peroxide sensor based on the redox of ferrocene carboxylic acid on ionic liquid film-modified screen-printed graphite electrode

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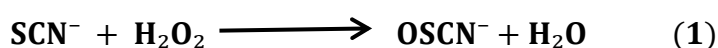
Abstract

Globally, the use of H₂O₂ for the preservation of raw milk has a long established history. However, in the EU, US and most parts of the world, where access to refrigeration facilities is widely available, the adulteration of milk with H₂O₂ is generally not permitted. An in-house hand-printed carbon electrode consisting of graphite printing ink modified with the room temperature ionic liquid (RTIL), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF₄]), ferrocene carboxylic acid (Fca) and cellulose acetate (CA) for the electrochemical sensing of hydrogen peroxide (H₂O₂) in commercially packaged aseptic milk is described. The developed electrode successfully enabled sensitive determination of H₂O₂, free from interference from some known electroactive species such as ascorbic acid (AA), dopamine (DA), glucose and uric acid (UA). The linear range for the determination of H₂O₂ was 1.0 µM - 1.2 mM with a limit of detection of 0.35 µM and a sensitivity of 10.6 nAµA⁻¹µM⁻¹cm⁻². When used for the analysis of H₂O₂ residues in milk samples, the resulting precision (n = 6) and recovery were 0.53 % and 97.8 %, respectively.

Keywords: Ionic liquid, hydrogen peroxide, screen-printed electrode, ferrocene, milk, cellulose acetate

1. Introduction

The determination of hydrogen peroxide (H_2O_2) is of great relevance in the food and beverage industry; thus, creating the need for the fabrication of easy-to-use, selective, sensitive, and single-use disposable H_2O_2 sensors [1-4]. H_2O_2 has inherent bactericidal and sporicidal properties and is often used to clean mixing, bottling, transporting and packing equipment in the food industry [2,3]; hence, H_2O_2 may become incorporated into these surfaces and require an additional processing step to remove or decompose it. Its bactericidal and sporicidal properties are based on the fact that fresh raw milk contains the enzyme lactoperoxidase (which has no antibacterial properties on its own) but has the ability to catalyse the oxidation of inherent thiocyanate (SCN^-) in the presence of H_2O_2 (eqn 1) [2];



The resulting chemical compound (OSCN^-) has an antibacterial effect in fresh raw milk. Hence, H_2O_2 is often used to activate this inherent lactoperoxidase enzyme system (shown in equation 1) to preserve raw milk dairy particularly in areas where refrigeration is not widely available [3-5]. However, against some species of the normal flora of human gut including *streptococci* and *lactobacilli*, the compound OSCN^- has a bacteriostatic effect (inhibition of bacterial growth). Consequently, the ingestion of milk containing residues of H_2O_2 can cause gastrointestinal problems [6]. H_2O_2 in milk can also cause the degradation of vitamins (e.g. folic acid) [7] and some essential amino acids such as methionine [8] resulting in a reduction of its nutritional value. Therefore, the use of H_2O_2 for the preservation of raw milk within the EU, United States, and most parts of the world, where refrigeration is widely available, is not permitted except in certain applications such as cheese-making and modified whey [1, 5].

Currently, the measurement of H_2O_2 in the food and beverage industry includes spectroscopy, [9, 10] and enzyme-based fluorescence or chemiluminescence assays [11, 12]. However, these methods are relatively expensive, require special storage facilities to preserve the enzyme activities, cumbersome and often require that samples are pre-treated prior to their analysis thus, rendering them unsuitable for routine analysis of H_2O_2 . Owing to the electroactive nature of H_2O_2 , the use of electrochemical sensors are most attractive for H_2O_2 analysis in milk [13, 14] because they are rapid, simple, relatively less expensive, selective and sensitive, and allows for direct real-time and online data analysis that excludes sample pre-treatment procedures [15, 16]. But the main drawbacks associated with traditional electrode materials for electrochemical measurements are low reproducibility due to electrode fouling and the poor selectivity arising from common interfering species such as DA, AA, and UA. Electrodes modified with redox mediators [15] and perm-selective membranes [16-18] have been useful in solving these problems. Consequently, there is the need for the development of enzyme-free methods for H_2O_2 analysis.

Typically, the use of mediators can allow H_2O_2 to be detected at lower potentials, thus substantially reducing the interfering influence of other electroactive species [17]. However, the immobilisation of redox mediators can be problematic as it can be poorly adsorbed onto such electrodes [15]. One possible route to solve problems from interferences is to use perm-selective membranes in conjunction with a redox mediator [18], thereby excluding the access of many interfering species to the surface of the electrode.

Room temperature ionic liquids (RTILs), salts that are liquid at room temperature, are generally considered to be 'green solvents' and have been used as catalytic supports [19]. Consequently, in the current study, the RTIL, 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF₄]) was used as a 'green support' for the immobilisation of a redox mediator, ferrocene carboxylic acid (Fca) onto the working area of a screen-printed graphite electrode

(SPGE). Additionally, cellulose acetate (CA), serving as a perm-selective membrane, was incorporated into the sensor design to act as an exclusion barrier. Overall, the specific advantages of screen-printed platforms (miniaturisation and low-cost) coupled with the use of RTIL in conjunction with the perm-selective membrane, CA to immobilise the redox mediator, Fca are assembled to fabricate a simple, selective and sensitive sensor for the quantification of H_2O_2 in commercially packaged aseptic milk; thus, providing an alternative in routine analytical sensing of milk adulteration. Details of the sensor fabrication and characterisation are described and discussed.

2. Experimental

2.1 Apparatus and reagents

Electrochemical experiments were conducted using VSP-300 Multichannel Potentiostat/Galvanostat/EIS (Bio-Logic Science Instruments, France) with a standard three-electrode configuration. The SPGE were hand-printed using a Stainless Steel Screen Mesh (DEK: 159784, ASM Assembly Systems). Valox substrate was purchased from Cardillac Plastics, UK. A Ag/AgCl (1.0 M KCl) reference electrode was used throughout. The working electrode was SPGE with a platinum wire as the counter electrode. The room temperature ionic liquid 1-ethyl-3-methyl imidazolium tetrafluoroborate ([EMIM][BF₄]), cellulose acetate, acetone, catalase, ethanol were obtained from Sigma Aldrich. Ascorbic acid, uric acid and ferrocene carboxylic acid were purchased from Alfa Aesar, UK. All other chemicals were of analytical grade and used without further purification. Commercially packaged aseptic milk samples were obtained from a local convenience store.

2.2 Preparation and analytical application of the sensor

The base unmodified SPGE transducer was prepared using graphite ink (GEM Product code: C205010697) and the sensors were screen-printed in groups of eight onto valox substrate and cured at 70 °C for 90 minutes. The SPGE was modified by drop-coating 5 μ l of 1.5% CA-acetone solution directly onto the working area (3.5 x 3.5 mm) of the electrode to form CA-SPGE. The presence of CA was crucial for forming a stable RTIL film. Once dry, the electrode was exposed to 10% RTIL-ethanol solution for 1hr to form RTIL-CA-SPGE. Lastly, the RTIL-CA-SPGE was immersed in 5 mM ferrocene carboxylic acid (Fca) solution for about 24hrs to form Fca-RTIL-CA-SPGE. In addition to this, Fca-CA-SPGE, Fca-RTIL-SPGE and Fca-SPGE sensors were fabricated in a similar fashion. Prior to each measurement, all solutions were degassed in with N₂ for about 10 minutes. Once prepared, the sensors were placed in Britton-Robinson buffer (pH 7.0) and stored at 4 °C overnight.

The milk samples were prepared by mixing 5 ml of milk in 25 ml of Britton-Robinson buffer (pH 7.0) and spiked with 500 μ M H₂O₂. Prior to this, the enzyme catalase was used to verify whether H₂O₂ was present in the milk samples. For the recovery studies, aliquots (2 ml) of the spiked milk samples were placed in the electrochemical cell containing 3 ml BR buffer (pH 7.0) and subjected to amperometry at an applied potential of +0.5 V until a steady baseline was obtained. Thereafter, multiple standard additions of H₂O₂ were added to the electrochemical cell under stirred conditions and a steady state current response obtained. The difference between the baseline and the steady state current for H₂O₂ additions was used to calculate the recovery of the added H₂O₂. All the measurements were repeated (n = 6).

3. Results and discussion

3.1 Electrochemical behaviour of Fca-RTIL-CA-SPGE electrode

Figure 1A shows cyclic voltammograms of Fca-CA-SPGE and Fca-RTIL-CA-SPGE electrodes after overnight storage in BR buffer (pH 7.0). A pair of reversible redox peaks could be observed on Fca-RTIL-CA-SPGE electrode. The anodic and cathodic peak potentials were located at 0.371 and 0.309 V, respectively and the peak separation (ΔE_p) was found to be ~62 mV; which is considerably close to 59 mV value expected for Nernstian one electron reactions [20]. However, very weak peaks (not shown) could be observed when Fca-CA-SPGE, Fca-RTIL-SPGE and Fca-SPGE electrodes were used immediately following fabrication and no redox peaks were observed after overnight storage in buffer solution. This indicates that the Fca was only loosely bound onto the CA membrane and bare SPGE electrode.

The result also indicates that CA membrane is crucial for the formation of a stable RTIL film. There was no apparent change in redox peaks on the Fca-RTIL-CA-SPGE electrode before and after overnight storage in buffer; this proved that the Fca was stably adsorbed onto the surface of the SPGE by the RTIL film. At the scan rates investigated using Fca-RTIL-CA-SPGE electrode, both the anodic and cathodic potentials remained unchanged (Figure 1B) and the peak currents increased with increasing scan rates (Figure 1C); indicating that the electrochemical reaction is a mixture of diffusion and adsorption-controlled processes and correspond to the electrochemistry of the deposited film [21]. Assuming the condition of saturated adsorption of Fca and using the Laviron's equation [22], $I_p = n^2 F^2 \nu A \Gamma / 4RT$ [where ν is the scan rate, A is the electrode surface area (here 0.1225 cm^2) and the other symbols have their usual meaning], the average surface coverage of Fca (for scan rates $> 100 \text{ mVs}^{-1}$) on the RTIL film was estimated to be $(2.01 \pm 0.05) \times 10^{-7} \text{ molcm}^{-2}$ for $n = 1$. This coverage value is in general agreement with adsorption of ferrocene on nafion-RTIL layers [23].

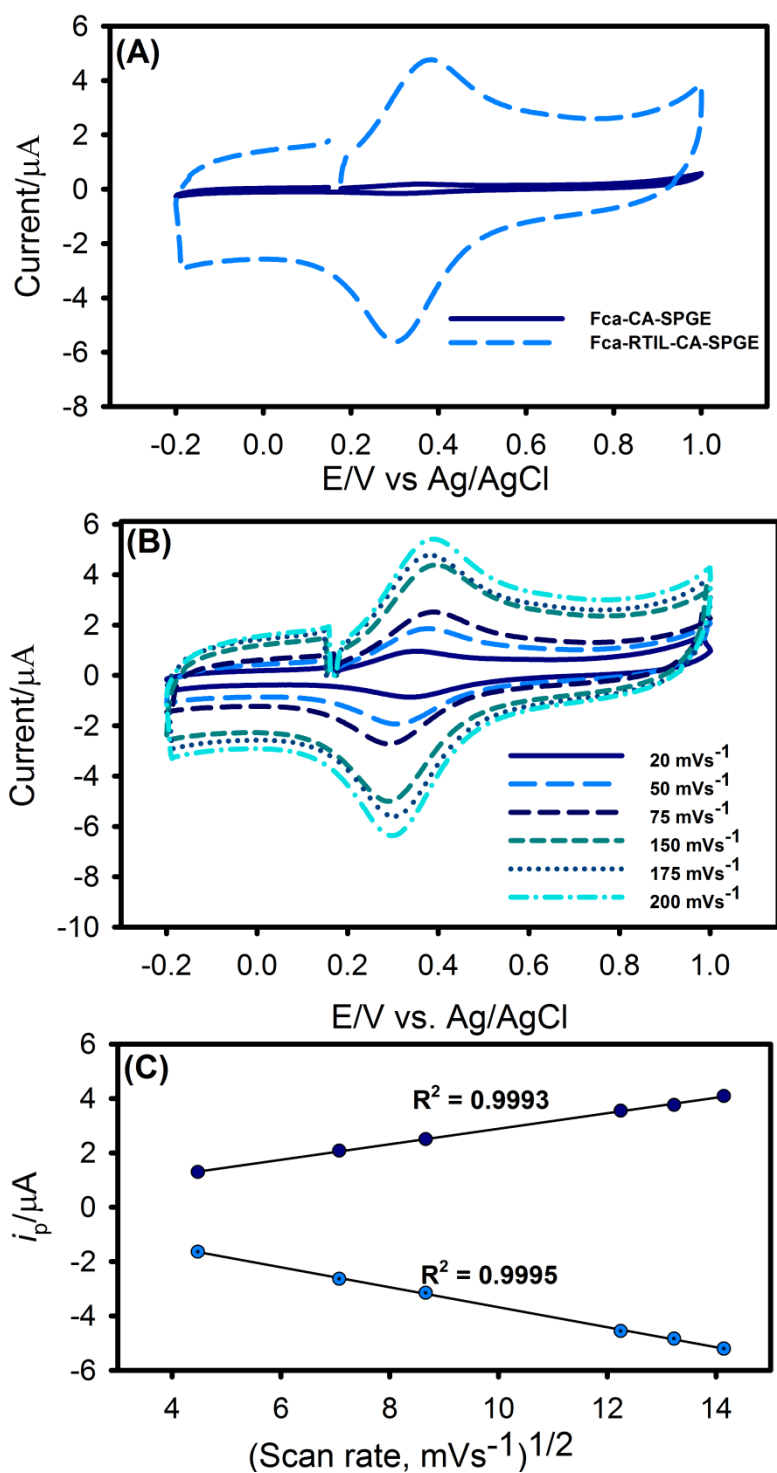


Figure 1: Cyclic voltammograms obtained at (A) Fca-CA-SPGE and Fca-RTIL-CA-SPGE in Britton-Robinson buffer (pH 7.0) after overnight storage at 4 °C, 175 mVs^{-1} scan rate; (B) Fca-RTIL-CA-SPGE in Britton-Robinson buffer (pH 7.0) at scan rates of 20, 50, 75, 150, 175 and 200 mVs^{-1} . All buffer contained 0.1 M KCl solution; (C) plot of I_p vs. \sqrt{V} .

Cyclic voltammograms of Fca-RTIL-CA-SPGE electrode with and without H_2O_2 is shown in Figure 2. Both anodic and cathodic peak currents increase with increase H_2O_2 concentration. These results suggest that the ferrocene adsorbed onto the Fca-RTIL-CA-SPGE electrode catalyse the oxidation of H_2O_2 to O_2 or reduction to H_2O [24] and that the RTIL film confined on the SPGE efficiently promotes the direct electron transfer of the ferrocene.

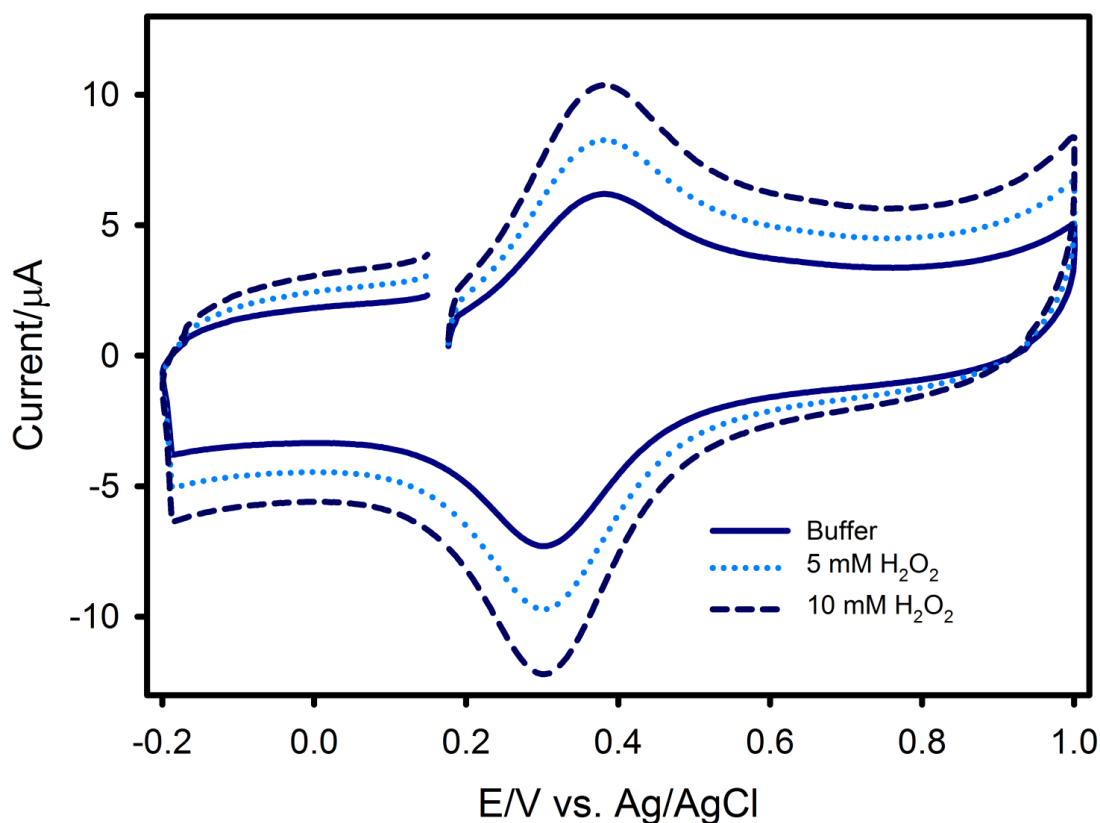


Figure 2: Cyclic voltammograms obtained at Fca-RTIL-CA-SPGE electrode for 5 mM and 10 mM H_2O_2 in Britton-Robinson buffer (pH 7.0) containing 0.1 M KCl. Scan rate: 175 mVs^{-1} .

Hence, the proposed mechanism for these reactions is that both the oxidation of H_2O_2 to O_2 and the reduction of H_2O_2 to H_2O can be catalysed by the redox couple of Fca^+/Fca (Figure 2). To verify this, the sensor was placed in the electrochemical cell containing degassed BR buffer (pH 7.0) solution and a potential of +0.5 V was applied, until steady state current was

obtained (Figure 3A). Following the establishment of steady state current, 0.5 mM H_2O_2 was added to the solution (under stirred conditions) and this elicited a rapid oxidation current response which can be attributed to the added H_2O_2 . This experiment was repeated using an applied potential of -0.4 V. As can be seen in Figure 3B, the addition of 0.5 mM H_2O_2 elicited a rapid reduction current response, attributable to the reduction of the added H_2O_2 . This behaviour illustrates that the ferrocene carboxylic acid adsorbed onto the surface of the proposed electrode could catalyse the oxidation of H_2O_2 to O_2 at +0.5 V and the reduction of H_2O_2 to H_2O at -0.4V as has been observed elsewhere [23]. Consequently, the Fca-IL-CA-SPGE electrode could be used as a sensor for the quantification of H_2O_2 .

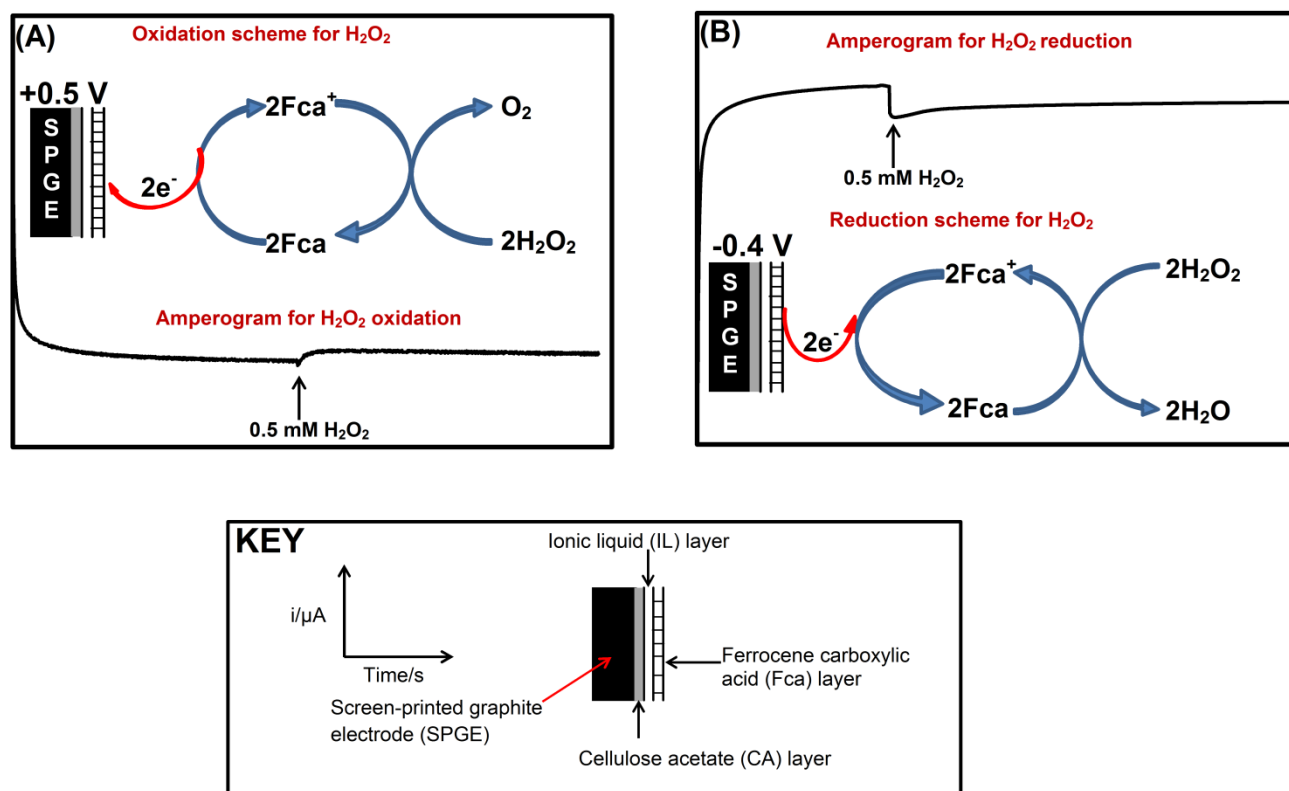


Figure 3: Amperometric response of Fca-IL-CA-SPGE to 0.5 mM H_2O_2 with their corresponding reaction mechanism schemes for the (A) oxidation of H_2O_2 to O_2 at +0.5 V and (B) reduction of H_2O_2 to H_2O at -0.4V. H_2O_2 was prepared in Britton-Robinson buffer (pH 7.0).

3.2 Calibration, Fca stability and interference studies on Fca-RTIL-CA-SPGE electrode

Standard additions of H_2O_2 were performed with the Fca-RTIL-CA-SPGE electrode over the range of 0.1-1200 μM at an applied potential of 0.5 V. Figure 4A displays a typical response of the electrode to standard concentrations of H_2O_2 . Each H_2O_2 addition elicited a rapid current response and the resulting calibration plot (Figure 4B) exhibited a slope of $1.3 \text{ nA}\mu\text{M}^{-1}$ ($10.6 \text{ nA}\mu\text{M}^{-1}\text{cm}^{-2}$), linearity of 1-1200 μM and a calculated limit of detection (based on 3x the baseline noise) of 0.35 μM . These electroanalytical performance characteristics of the Fca-RTIL-CA-SPGE electrode are superior to published studies obtained from similar RTIL-modified surfaces (Table 1), which tended to have reduced linear range [23, 25, 26, 27, 28] and/or poor detection limits [23, 28, 29].

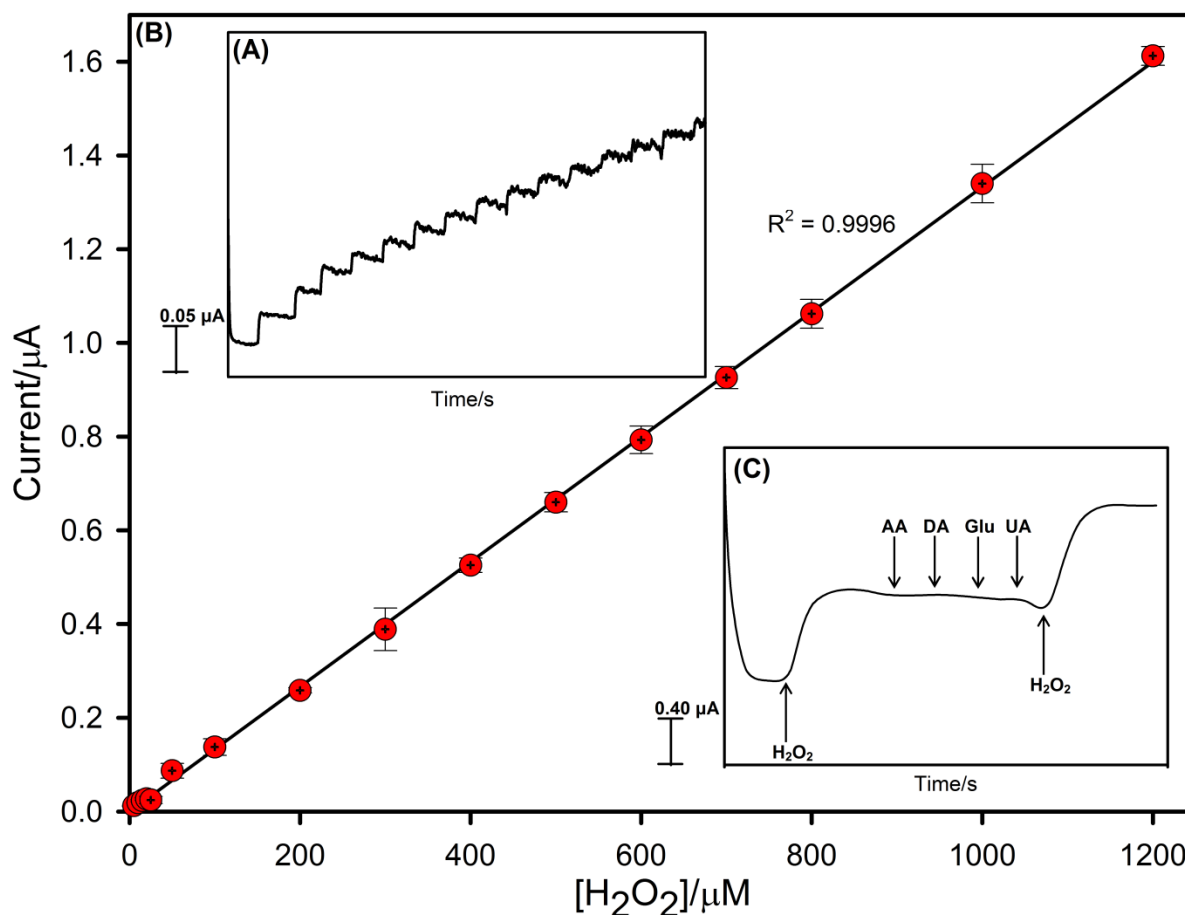


Figure 4: (A) Typical current-time response of the electrode to successive standard additions of H_2O_2 into stirred solution of Britton-Robinson buffer (pH 7.0) at + 0.5V; (B) Calibration curve of the Fca-RTIL-CA-SPGE. Error bars represent the standard deviation for three independent measurements; (C) Interference study by successive additions of 2 mM each of ascorbic acid (AA), dopamine (DA), glucose (Glu) and uric acid (UA), and 1 mM H_2O_2 in Britton-Robinson buffer (pH 7.0) containing 0.1 M KCl at + 0.5 V (vs. Ag/AgCl).

Under the experimental conditions, 20 continuous cyclic voltammograms were carried out at 175 mVs^{-1} scan rate and no obvious change of the peak currents on the Fca-RTIL-CA-SPGE electrode could be observed after 14 days storage in BR buffer (pH 7.0). Four possible interfering species ascorbic acid (AA), dopamine (DA), glucose and uric acid (UA) were investigated. Figure 4C showed that 2 mM each of ascorbic acid and uric acid could not elicit

any response from the electrode. This showed that cellulose acetate has the dual effect of serving as a support for RTIL film and excluding the direct oxidation of these interferences as previously reported [18].

Table 1: Comparison of different RTIL-modified electrodes for H₂O₂ detection

Electrode	Linear range (μM)	LoD (μM)	Reference
Fc-Nafion-[BMIM][BF ₄]-BPGE	-	50	[23]
HRP-chi-[BMIM][BF ₄]-GCE	0.75 – 135	0.25	[25]
HRP-[BMIM][BF ₄]-Chi-Au	0.6 - 160	0.15	[26]
Cyt c-[2-MBPy][BF ₄]-BPGE	0 – 16	0.05	[27]
Mb-[BMIM][BF ₄]-HA-GCE	2.0 - 270	0.6	[28]
Cyt c-AuNps-[BMIM][BF ₄]-CNTs-GCE	50 - 1150	3.0	[29]
Fca-[BMIM][BF ₄]-CA-SPGE	1.0 – 1200	0.35	this study

LoD. Limit of detection; [EMIM][BF₄], 1-ethyl-3-methylimidazolium tetrafluoroborate; [2-MBPy][BF₄], 2-methyl-N-butylpyridinium tetrafluoroborate; HRP, horseradish peroxidase; Chi, chitosan; Cyt c, cytochrome c; AuNps, gold nanoparticles; BPGE, basal plane graphite electrode; CNTs, carbon nanotubes; GCE, glassy carbon electrode; HA, hyaluronic acid

3.3 Analytical application of the sensor

Prior to this analysis, 5.0 U/l of the enzyme catalase was injected into the diluted milk sample; this was to verify the presence (or otherwise) of endogenous H₂O₂ in the milk samples. As depicted in Figure 5A, there was no change in amperometric current response following the addition of the catalase, which indicates that there are no H₂O₂ residues in the milk. After verifying the absence of endogenous H₂O₂ in the milk, amperometry, in conjunction with the method of standard additions, was employed to determine the recovery of H₂O₂ spiked into the commercially packaged aseptic milk. Figure 5B shows the typical amperogram on the determination of H₂O₂ in the milk sample and Table 2 shows the results

obtained. They show a value of 97.8 % for the recovery of H_2O_2 , with coefficient of variation (CV) of 0.54 %. This data demonstrates that the sensor could be employed for the detection of H_2O_2 adulteration in packaged milk; especially in countries where the use of H_2O_2 as a stabiliser in milk is prohibited.

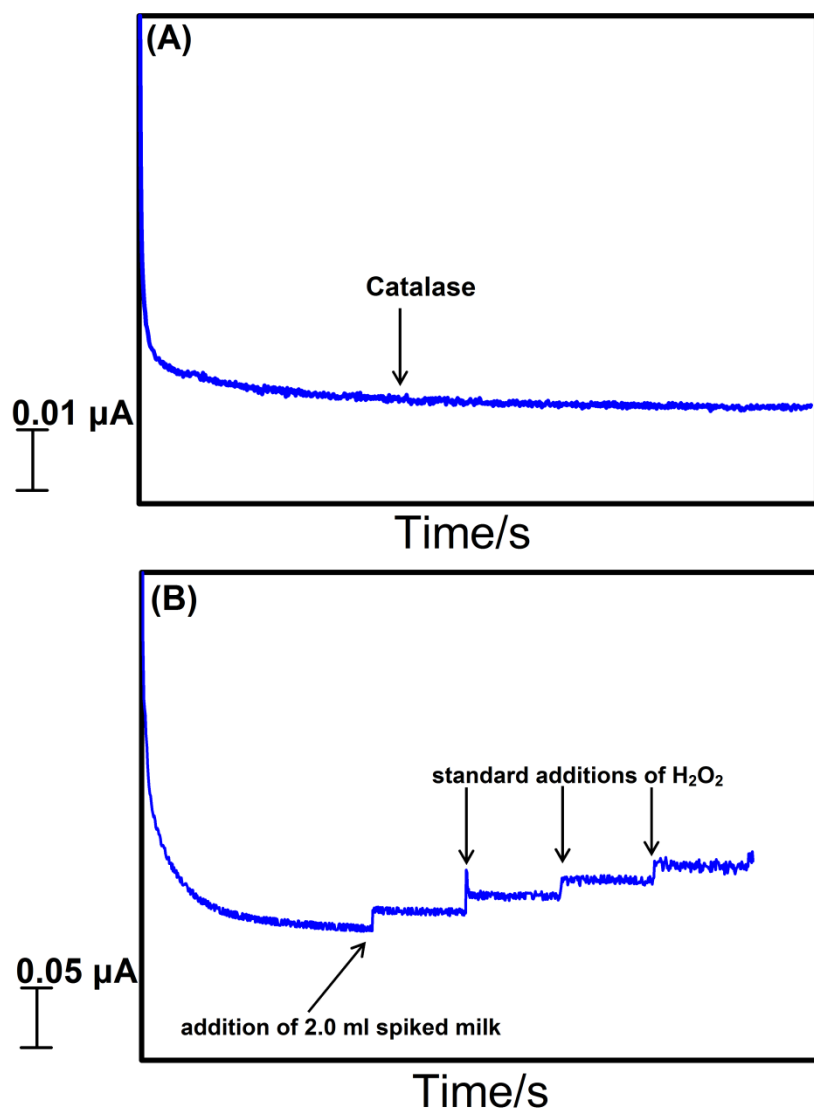


Figure 5: (A) Amperometric response of the Fca-RTIL-CA-SPGE in commercially packaged aseptic milk before and after the addition of 5.0 U/l of the enzyme catalase at an applied potential of +5.0 V; (B) Amperometric response of the Fca-RTIL-CA-SPGE towards 200 μM H_2O_2 spiked milk sample plus multiple standard additions of H_2O_2 at +0.5 V.

Table 2: The recovery of H₂O₂ from commercial aseptic milk samples

	[H ₂ O ₂]/μM		Mean Recovery (%)
<i>Sample</i>	<i>Amount Added</i>	<i>Amount Found*</i>	
1	200	194.9	$\%Recovery = \frac{(195.6)}{200} \times 100 = 97.8$
2	200	195.9	
3	200	196.8	
4	200	195.2	
5	200	194.1	
6	200	196.7	
Mean	-	195.6	
SD	-	1.06207	
CV (%)	-	0.543	
*the mean value of six measurements; SD- standard deviation; CV- coefficient of variation			

4. Conclusions

With the aid of RTIL and cellulose acetate, ferrocene carboxylic acid can be strongly adsorbed onto the surface of screen-printed graphite electrodes. The adsorbed ferrocene carboxylic acid can be used as an excellent redox species to mediate electron transfer and the oxidation of H₂O₂ can be catalysed by the ferrocene. The incorporation of cellulose acetate into the electrode design enabled the exclusion of interfering electroactive species. Thus, this fabricated Fca-RTIL-CA-SPGE electrode can be utilised as a sensing platform for sensitive detection of H₂O₂ in complex media. When used for the analysis of H₂O₂ residues in commercially packaged aseptic milk samples, the resulting precision (n = 6) and recovery were 0.53 % and 97.8 %, respectively. In the future, screen-printed microarray

biosensors/immunosensors, based on the transduction capabilities of Fca-RTIL-CA-SPGE, for multiplexed detection of a variety of protein biomarkers would be developed. This has the additional potential for personalised treatment of human diseases.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Hsu C-L, Chang K-S, Kuo, J-C. Determination of hydrogen peroxide residues in aseptically packaged beverages using amperometric sensor based on a palladium electrode. *Food Control* 2008; 19:223-230.
2. Haddadin MS, Ibrahim SA, Robinson RK. Preservation of raw milk by activation of the natural lactoperoxidase systems. *Food Control* 1996; 7:149-152.
3. Fox PF, Kosikowski FV. Heat-treated and hydrogen treated milks for cheddar cheese. *J of Dairy Science* 1962; 45:648-652.
4. Toledo RT. Chemical sterilants for aseptic packaging. *Food Technol* 1975;29:5.
5. Martin NH, Friedlander A, Allen M, Kent D, Weidmann M, Boor KJ. Peroxide test strips detect added hydrogen peroxide in raw milk at levels affecting bacterial load. *J Food Protection*, 2014; 77:1809-1813.

6. Silva RAB, Montes RHO, Richter EM, Munoz RAA. Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection. *Food Chemistry* 2012; 133:200-204.
7. Taher MM, Lakshmaiah N. Folic acid stability in hydrogen peroxide-potassium thiocyanate-treated milk. *Food Chemistry* 1992; 44:343-347.
8. Gregory ME, Henry KM, Kon SK, Porter JWG, Thompson SY. The effect of hydrogen peroxide on the nutritive value of milk. *J Dairy Res* 1961; 28:177.
9. Nogueira RFP, Oliverira MC, Paterlini WC. Simple and fast spectrophotometric determination of H₂O₂ in photo-fenton reactions using metavanadate. *Talanta* 2005; 66: 86-91.
10. Abbas ME, Luo W, Zou J, Tang H. Fluorimetric determination of hydrogen peroxide in milk by using Fenton reaction system. *Food Chem* 2010; 120:327-331.
11. Juven BJ, Pierson MD. Antibacterial effects of hydrogen peroxide and methods for its detection and quantification. *J of Food Protect* 1996; 59:1233-1241.
12. Alain CC, Spoon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20:470-475.
13. Alpat S, Alpat KS, Dursun Z, Telefoncu A. Development of a new biosensor for mediatorless voltammetric determination of hydrogen peroxide and its application in milk samples. *J of Appl Electrochem* 2010; 39:971-977.
14. Campuzano S, Pedrero M, Pingarron JM. A peroxidase-tetrathiafulvalene biosensor based on self-assembled monolayer modified Au electrodes for the flow-injection determination of hydrogen peroxide. *Talanta* 2005; 66:1310-1319.
15. Chaubey A, Malhotra BD. Review- mediated biosensors. *Biosens Bioelectron* 2002; 17:441-456.

16. Hart JP, Crew A, Crouch E, Honeychurch KC, Pemberton RM. Some recent designs and developments of screen-printed carbon electrochemical sensors/biosensors for biomedical, environmental and industrial analyses. *Anal Letts* 2005; 37:789-830.
17. Kanyong P, Pemberton RM, Jackson SK, Hart JP. Development of an amperometric screen-printed galactose biosensor for serum analysis. *Anal Biochem* 2013; 435:114-119.
18. Kanyong P, Pemberton RM, Jackson SK, Hart JP. Development of a sandwich format, amperometric screen-printed uric acid biosensor for urine analysis. *Anal Biochem* 2013; 428:39-43.
19. Marsh KN, Boxall JA, Lichtenthaler R. Room temperature ionic liquids and their mixtures- a review. *Fluid Phase Equilibria* 2004; 219:93-98.
20. Ito N, Saji T, Aoyagui S. Electrochemical formation of stable ferrocene anion and the formal rate constant of ferrocene electrode. *J Organomet Chem* 1983; 247:301-5.
21. Murray RW, in: A. J. Bard (ed), *Electroanalytical chemistry*, vol. 13, Marcel Dekker, New York, 1984, pp. 191-368.
22. Laviron E. The use of linear potential sweep voltammetry and a.c. voltammetry for the study of the surface reaction of strongly adsorbed systems and redox modified electrodes. *J Electroanal Chem Interfac Electrochem* 1979; 100:263-70.
23. Zhao G-C, Xu M-Q, Zhang Q. A novel hydrogen peroxide sensor based on the redox of ferrocene on room temperature ionic liquid film. *Electrochem Commun* 2008; 10:1924-6.
24. Chang J-L, Wei G-T, Zen J-M. Screen-printed ionic liquid/preanodised carbon electrode: effective detection of dopamine in the presence of high concentration of ascorbic acid. *Electrochem Commun* 2011; 13: 174-7.
25. Lu X, Zhang Q, Zhang L, Li J. Direct electron transfer of horseradish peroxidase and its biosensor based on chitosan and room temperature ion liquid. *Electrochem Commun* 2006; 6:874-878.

26. Xi F, Liu L, Wu Q, Lin X. One-step construction of biosensor based on chitosan-ionic liquid-horseradish peroxidase biocomposite formed by electrodeposition. *Biosens Bioelectron* 2008; 24:29-34.
27. Ding S-F, Wei W, Zhao G-C. Direct electrochemical response of cytochrome c on a room temperature ionic liquid, N-butylpyridinium tetrafluoroborate modified electrode. *Electrochem Commun* 2007; 9:2202-2206.
28. Zhang Y, Zheng J. Direct electrochemistry and electrocatalysis of myoglobin immobilised in hyaluronic acid and room temperature ionic liquid composite film. *Electrochem. Commun* 2008; 10:1400-1403.
29. Xiang C, Zou Y, Sun L-X, Xu F. Direct electron transfer of cytochrome c and its biosensor on gold nanoparticles/room temperature ionic liquid/carbon nanotubes composite film. *Electrochem Commun* 2008; 10:38-41.